

**EARLY DEVELOPMENT OF  
*PSEUDOPHOXINUS STYMPHALICUS* (CYPRINIDAE)  
FROM LAKE TRICHONIS, GREECE**

by

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**ABSTRACT.** - A developmental series of free embryos and larvae of the endemic cyprinid species *Pseudophoxinus stymphalicus* from Lake Trichonis (Greece) is described and illustrated, based on material from laboratory rearing of embryos hatched from wild-caught eggs. Morphological changes and the occurrence of important ontogenetic events are followed up to the time of metamorphosis. Characters of potential systematic value are indicated. The larvae are characterized by robust body shape, dark appearance and very weak development of the mediolateral row of melanophores, and immediately distinguishable from the other cyprinid larvae of the lake.

**RÉSUMÉ.** - Le développement des larves du cyprinidé endémique de Grèce *Pseudophoxinus stymphalicus* provenant du lac Trichonis est établi à partir d'élevages au laboratoire. Les changements morphologiques et les événements ontogénétiques importants ont été suivis jusqu'à la métamorphose, et des caractères à valeur systématique potentielle sont indiqués. Les larves sont très caractéristiques par la forme robuste du corps, leur couleur sombre et le développement très faible des mélanophores de la ligne médiolatérale. Ils peuvent être distingués immédiatement des larves d'autres cyprinidés.

**Key-words.** - Cyprinidae, *Pseudophoxinus stymphalicus*, Greece, Free embryos, Larvae, Development.

Greek freshwater bodies are inhabited by a large number of endemic taxa whose life-histories are poorly known. *Pseudophoxinus stymphalicus* (Valenciennes, 1844) is one of the least well known cyprinids. This species occurs among aquatic vegetation in marshes, quiet parts of lakes, slow-flowing riverine sites, and generally stagnant waters. Little is known about its relationships within the family Cyprinidae. Initially, it was classified in the genus *Leucaspis* (Stephanidis, 1939) and later was placed in the genus *Phoxinellus* (*Pararhodeus*) (Stephanidis, 1971, 1974). Now, it is recognized as a species of the genus *Pseudophoxinus* with three endemic subspecies in Greece and a fourth in Lake Ochrid of southern Yugoslavia (Economidis, 1991).

Localized populations occur in southern and western Greece. In Lake Trichonis, *P. stymphalicus* reaches a maximum size of 65 mm FL. Aspects of its biology and reproduction and a brief description of its larvae have been presented elsewhere (Economou *et al.*, 1994). Here, we provide a more complete developmental series of the free embryos and larvae of *P. stymphalicus*, focussing particularly on diagnostic characters allowing distinction from other co-occurring species of the Cyprinidae.

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## MATERIAL AND METHODS

On 15 February 1990, fertilized fish eggs firmly adhered to weeds were collected from shallow water (1 m depth) in a small fishing harbour of Lake Trichonis (water temperature was 13.5 °C) and were transported to the laboratory for incubation and rearing of larvae. At collection, the eggs were at various but early developmental stages (embryonic axis not established) and took 5 to 7 days to hatch in the laboratory at 19 °C. All embryos that hatched over an interval of 24 h on the sixth day from collection were placed for rearing in a 25 l aerated aquarium, and were fed ad libitum on artificial diets (dried *Daphnia* and flakes for fry fish), and occasionally on nauplii of brine shrimp. Because the experiment took place in a working room, the temperature was not specifically controlled, and increased throughout the experiment from 19 to 24 °C. Up to the time of metamorphosis, samples of free embryos and larvae were taken at 24 or 48 h intervals and were preserved in 4% formalin buffered with sodium phosphate. Due to that the reared population consisted of individuals which differed in age up to 24 h at the initiation of the experiment, bias is inevitably introduced in the ontogenetic descriptions in relation to age. Surviving juveniles beyond metamorphosis were reared to the age of 90 days, when then were killed and preserved in formalin. These juveniles were kindly identified for us as *P. symphalicus* by Professor P. Economidis of the University of Thessaloniki, based on adult characters.

Morphological development of free embryos and larvae was followed with the use of a stereomicroscope. Illustrations were drawn with the aid of a camera lucida. Lengths are given as notochord length (NL) for preflexion individuals and as standard length (SL) for flexion and postflexion ones. Line drawings and lengths were based on specimens kept in preservation for about six months. No allowance for shrinkage was made.

## RESULTS

*Pseudophoxinus symphalicus* lays down adhesive, semitransparent, yellowish spherical eggs ( $1.3 \pm 0.05$  mm,  $N = 20$ ) with a narrow perivitelline space on aquatic plants. Spawning in Lake Trichonis occurs between December and April, peaking in February and March, as inferred from the presence of free embryos in harbours and protected bays.

A developmental series from free embryos to juveniles of 12.7 mm in length is illustrated in figures 1-4. The embryos hatch at approximately 4.7 mm NL without a functional swimbladder (Fig. 1a). The primordial fin is relatively narrow, the eyes are pigmented, the anus is behind the midpoint of the body and is open at hatching, and there are pectoral fin buds. On day 2, the sagitta becomes visible, the swimbladder fills with air and the larvae are capable of swimming freely in the water column (Fig. 1b). The yolk is absorbed on day 3 at a size range between 5.7 and 6.1 mm, although remnants may remain up to day 4. Between days 4 and 5 the first caudal fin rays appear.

Already at hatching, the pigmentation pattern is well developed and gives the larvae a dark appearance. It consists of numerous close-spaced dorsal and ventral stellate melanophores arranged in double rows, a thick array of head melanophores and scattered lateral melanophores. The mediolateral row of melanophores, present in most cyprinid larvae, is only rudimentary developed, if not absent. No important changes in the pigmentation pattern occurred during the yolk sac and early larval periods except the addition of new melanophores in various positions of the body (Fig. 2).



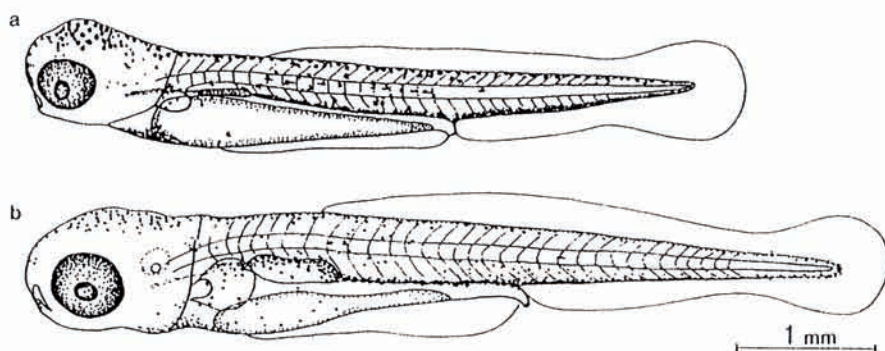


Fig. 1. - Free embryos of *Pseudophoxinus stymphalicus*. (a) 4.8 mm NL, day 1; (b) 5.8 mm NL, day 2.

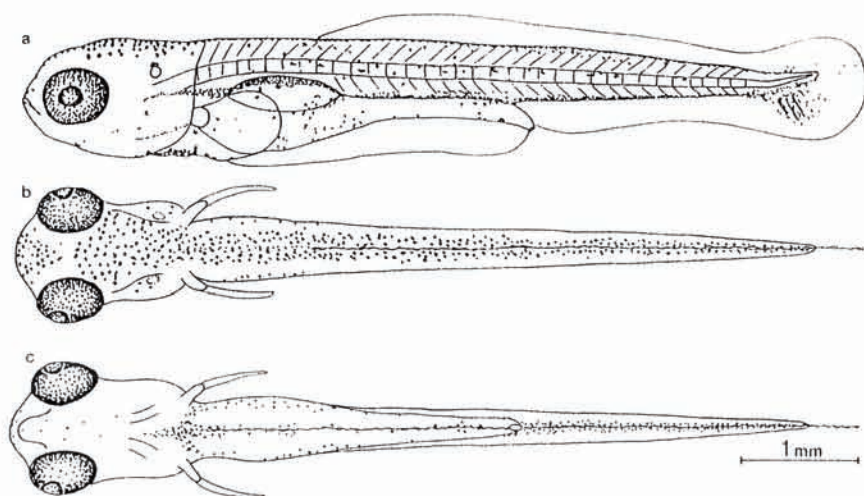


Fig. 2. - Larva of *Pseudophoxinus stymphalicus*, 6.7 mm NL, day 5; (a) lateral, (b) dorsal, (c) ventral view.

Notochord flexion is completed on days 9 to 11 at sizes between 6.9 and 7.4 mm (Fig. 3a), and immediately afterwards interspinous rays appear on the primordial fin in the positions of the future anal and dorsal fin development. Development of true anal and dorsal fin rays starts on days 12 to 15 at sizes between 7.7 and 8.0 mm (Fig. 3b). The body is by now very strongly pigmented and the pigment extends onto the pectoral, caudal, dorsal and anal fins.

Between days 15 and 20 the swimbladder becomes two-chambered. With further development, the primordial fin gradually disappears, except for a pigmented part in the abdominal region (Fig. 4a). Pelvic fin buds at around day 20 at 10.0-10.5 mm, and completion of pelvic ray development occurs by day 30 at around 11.5 mm. Transformation to the juvenile stage occurs around day 35 between 11 and 12 mm (Fig. 4b). By now, the fins

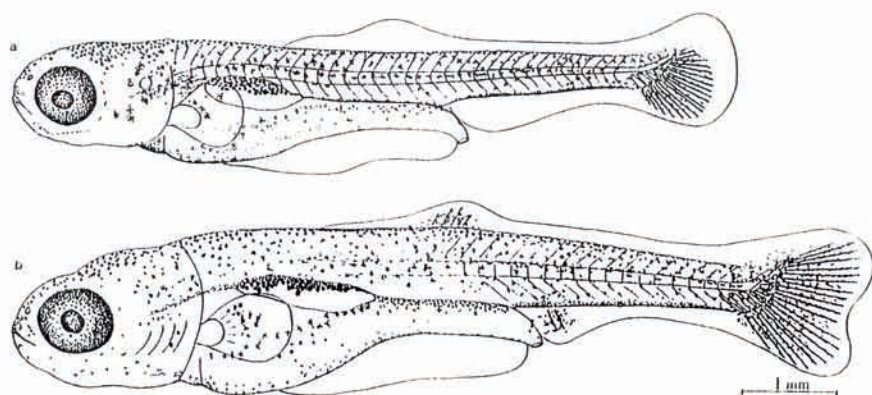


Fig. 3. - Larvae of *Pseudophoxinus stymphalicus*. (a) 7.1 mm SL, day 11; (b) 8.0 mm SL, day 15.

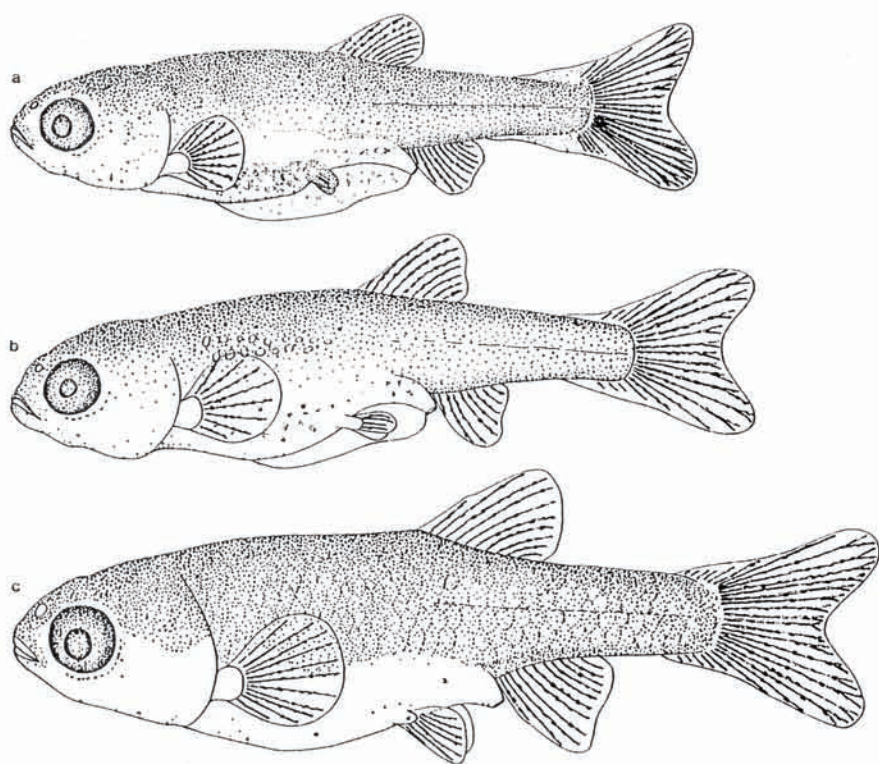


Fig. 4. - Larvae of *Pseudophoxinus stymphalicus* (a) 10.6 mm SL, day 22; (b) initiation of metamorphosis, 11.2 mm SL, day 35; (c) postmetamorphosed individual, 12.8 mm SL, day 42.

have taken their final form and coverage of the body with scales initiates, starting from the pectoral region and proceeding posteriorly (Fig. 4b, 4c).

From hatching to juveniles of around 18 mm, the ratio of the preanal to the standard length changes from 0.64 to 0.69. Over the same size interval the ratio of eye diameter to head length changes from 0.44 to 0.32. Already at the size of 8 mm, the body is more robust in comparison to that of other cyprinid larvae. Several characters, such as robustness, heavy pigmentation, melanophore distribution and the absence of a noticeable mediolateral row of melanophores, allow for distinction of this species from all other cyprinid larvae of the lake.

## DISCUSSION

Using the number of rows of melanophores along the body as a criterion of larval taxonomy, Koblitskaya (1981) classified the cyprinid larvae into three groups, one with three, one with two and one with one row of melanophores respectively. The almost complete absence of a mediolateral rows of melanophores in *Pseudophoxinus stymphalicus* larvae suggests the placing of this species into Koblitskaya's second group, which is represented in Lake Trichonis also by *Cyprinus carpio* L. and *Carassius auratus gibelio* (Bloch).

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